PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Collins et al.
Serial No. 10/604,022
Filed: June 23, 2003
For: MICROWAVE-ASSISTED PEPTIDE SYNTHESIS

Group Art Unit 1654
Examiner Marcela M. Cordero Garcia

DECLARATION OF DR. MICHAEL J. COLLINS:

1. My name is Michael J. Collins. I am the President and Chief Executive Officer of CEM Corporation ("CEM"), the assignee of Serial No. 10/604,022. I work at CEM's main place of business at 3100 Smith Farm Road, P.O. Box 200, Matthews, NC 28106-0200.

2. I received a Bachelor of Science degree in Chemistry, Cum Laude and Phi Beta Kappa, from the University of Florida in 1965. I also received a PhD in Physical Chemistry from the University of Texas in 1970, with an emphasis in microwave spectroscopy.

3. I began my professional career with Celanese Corporation, working in the research and development of synthetic fibers. During my eight years there, I also worked in sales and marketing.

4. I founded CEM Corporation in 1978. CEM manufactures instrumentation for analytical chemistry, process control, chemical synthesis and the biosciences. We sell our instruments throughout the world. In recent years, CEM's annual sales have exceeded $50,000,000 several times, and we expect sales to continue to increase.

5. I have received many entrepreneurial awards since the founding of CEM and in 1990, I was named North Carolina Entrepreneur of the Year by Inc. Magazine. I am a current member and former director of ALSSA (Analytical & Life Science Systems Association). I have had numerous publications in various trade journals and was a contributing author in a ACS Professional Reference Book entitled, INTRODUCTION TO MICROWAVE SAMPLE PREPARATION, THEORY AND PRACTICE. I am a named inventor on at least 28 issued U.S. patents and at least 23 published U.S. applications in the field of microwave technology and related instrumentation.

6. I am quite familiar with microwave instrumentation and analysis. Indeed, as a pioneer in this industry, CEM has originated many of the devices and test protocols used when incorporating microwave techniques into analytical chemistry.

7. I am a named coinventor of the invention described in Serial No. 10/604,022

8. Historically, because of the nature in which microwaves have been generated and propagated, microwave irradiation has been used for relatively robust types of reactions. Indeed, the development of microwave instrumentation in the various fields of chemistry was founded on relatively robust reactions such as microwave-driven drying of materials to calculate moisture content and the harsh digestion of materials in concentrated acids to prepare samples for further analysis such as atomic absorption and other analytical techniques.

9. For example, microwave-assisted acid digestion reactions can reach 300° C and generate pressures of up to 1500 psi in closed vessels.

10. As a result, microwaves have not represented a method of choice for heating or otherwise energizing certain reactions, and the use of microwaves for more sophisticated reactions requiring less energy and more precise control is a much more recent development.

11. In my opinion, the references attached to my declaration illustrate that conventional thinking encouraged the skilled person to avoid microwave irradiation (or other aggressive techniques) for deprotection steps such as those described and claimed in Serial No. 10/604,022.

12. The first reference is Yeh, *Microwave-Enhanced Liquid-Phase Synthesis of Thiohydantoins and Thioxotetrahydropyrimidinones*, Molecular Diversity, Vol. 7, pages 185-198 (2003). The authors experimented with microwave deprotection but were unable to carry it out successfully, and thus returned to room temperature deprotection instead.

> "Deprotection of the Fmoc group from compound 1 was attempted with microwave irradiation, but it was found that the amino acids were cleaved from the support. Instead, deprotection of compound 1 was performed with 10 percent piperidine in dichloromethane at room temperature for one hour."

(Page 186, right hand column, "Results and Discussion").

13. The second reference is Mergler, *The Aspatimide Problem in Fmoc-Based SPPS. Part I*, Journal of Peptide Science, Vol. 9, pages 36-46 (2003). These authors reported that elevated temperatures of as little as 45 degrees centigrade promoted side reactions and undesired byproducts, including undesired racemization; e.g., Table 2 and the "Results and Discussion" on page 41.

> "Harsher cleavage methods which may ensure complete deblocking for larger peptides, enhance the risk of aspartimide formation and other base-induced side reactions."

> "As expected, elevated temperatures, e.g. 45 degrees (see Table 2), promote the side reactions and also the stronger bases such as DBU or TMG give rise to a considerable amount of D/L- aspartimide and subsequent products."

14. In my opinion, prior to our invention, the conventional thinking as evidenced by these excerpts (but not necessarily limited to these examples) would discourage the skilled person from attempting to use microwave irradiation to carry out deprotection.

15. Both of the Yeh and Mergler articles describe deprotection of Fmoc-protected compounds. In my opinion, the same conventional reluctance to use microwaves or other aggressive techniques would also apply to compositions using other protective groups.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Michael J. Collins

Dr. Michael J. Collins

Date: 2/15/08

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JOURNAL OF PEPTIDE SCIENCE

The Aspartimide Problem in Fmoc-based SPPS. Part I†

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Abstract: A variety of Asp β-carboxy protecting groups, Hmb backbone protection and a range of Fmoc cleavage protocols have been employed in syntheses of the model hexapeptide H-VKDGYI-OH to investigate the aspartimide problem in more detail. The extent of formation of aspartimide and aspartimide-related by-products was determined by RP-HPLC. This study included three new Fmoc-Asp-OH derivatives: the β-(4-pyridyl-diphenylmethyl) and β-(9-phenyl-fluoren-9-yl) esters and also the orthoester Fmoc-β-(4-methyl-2,6,7-trioxabicyclo[2.2.2]-oct-1-yl)-alanine. 3-Methylpent-3-yl protection of the Asp side chain resulted in significant improvements with respect to aspartimide formation. Complete suppression was achieved using the combination OtBu side chain protection and Hmb backbone protection for the preceding Gly residue. Copyright © 2003 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: aspartimide formation; Fmoc-solid phase peptide synthesis; Asp β-carboxy protection; backbone protection; Asp-Gly motif; carboxy protection by orthoester formation

INTRODUCTION

Aspartimide formation is one of the best-documented side reactions in peptide synthesis. The sequence-dependent cyclization is catalysed by acids and by bases [1], and even bulky β-carboxy side-chain protecting groups such as OtBu do not prevent completely its occurrence. During Fmoc/*t*Bu-based SPPS, the repetitive piperidine treatments needed for Fmoc removal lead to successive formation of aspartimide. Further by-products result from racemisation of the imide derivative, which may also be opened by nucleophiles [2] as shown in Figure 1.

In previous studies, the hexapeptide fragment Val-Lys-Asp-Gly-Tyr-Ile (**I**) derived from scorpion toxin II [3] has been applied to investigate the susceptibility of the Asp-Gly motif for aspartimide formation. In this study, different protecting groups and a variety of conditions using model peptide **I** were investigated systematically. To address the problem of incomplete Fmoc removal, harsher conditions (stronger bases) for Fmoc deblocking were applied to verify the effect on aspartimide formation. The aim of this project was to use the resulting optimized combination of protecting groups and bases for the SPPS of longer peptides to improve the quality of the crude product. In addition to these systematic experiments, our study includes information on the synthesis of the following new Asp derivatives: Fmoc-Asp(OPyBzh)-OH, Fmoc-Asp(OPhFl)-OH and Fmoc-Asp(OBO)-OH.

Abbreviations: As recommended in *J. Peptide Sci.* 1999; **5**: 465–471, with the following additions and variations: LCMS, liquid chromatography coupled with mass spectrometry; *t*Bu, *t*-butyl; d, D-Asp; iPrOH, isopropanol; OMpe, 3-methylpent-3-yl ester; OPhFl, 9-phenyl-fluoren-9-yl ester; OPp, 2-phenylisopropyl ester; OPyBzh, 4-pyridyl-diphenylmethyl ester; Fmoc-Asp(OBO)-OH, Fmoc-β-(4-methyl-2,6,7-trioxabicyclo[2.2.2]-oct-1-yl)-alanine; TMG, 1,1,3,3-tetramethylguanidine.

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† A preliminary account of the present work was presented. Mergler M, Dick F, Sax B, Weiler P, Vorherr T. Systematic investigation of the aspartimide problem. *17th American Peptide Symposium, San Diego, USA, 2001* p63–64 M. Lebl and R. A. Houghten, eds.

MATERIALS AND METHODS

^{1}H-NMR measurements were performed on a Bruker Avance DRX 500 spectrometer, 500 MHz, employing

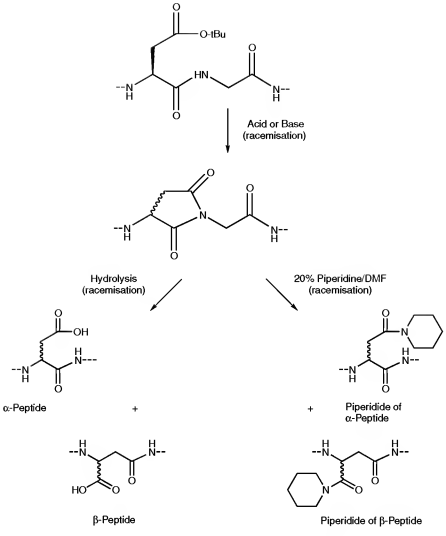


Figure 1 Formation of aspartimide and related products.

tetramethylsilane as an internal standard. ESIMS and LCMS-spectra were recorded in the positive mode with a Finnigan MAT LCQ mass spectrometer coupled to a Waters Alliance HPLC system. Analytical RP-HPLC-chromatograms were obtained employing a Merck-Hitachi chromatograph consisting of: pump L-6200, UV-detector L-4000, integrator D-2500, column thermostat L-5025. TLC-monitoring was performed applying silica gel plates Merck Kieselgel 60 F_{254} and the following systems for development: chloroform/MeOH/AcOH (90:8:2) (A), chloroform/32% aqueous AcOH/MeOH (15:4:1) (B), chloroform/AcOH/EtOAc (50:2:50) (C), chloroform/AcOH/EtOAc (90:2:10) (D); for detection: UV or KI/2-tolidine after oxidation with chlorine for general detection; ninhydrin for the presence or absence of free amino groups.

Fmoc-Asp(OPyBzh)-OBzl

Fmoc-Asp-OBzl (18.02 g, 40.4 mmol) was suspended in dry dichloroethane (100 ml) at room temperature yielding a sticky gel. PyBzh-Cl·HCl (10.45 g, 33.0 mmol), which was synthesized according to Coyle *et al.* [4], was added and the gel dissolved. A solution of DIPEA (12.6 ml, 73.6 mmol) in dichloroethane (13 ml) was added dropwise. The reaction was monitored by TLC (system A). The moderately basic solution slowly turned darker and slightly turbid. After stirring at room temperature

for 48 h, the solution was kept at 40 °C for 2 h, then the solvent was removed. The residue was taken up in water and extracted three times with EtOAc (500 ml each). Extraction removed only very polar impurities such as the DIPEA hydrochloride. Further aqueous washes did not remove excess Fmoc-Asp-OBzl, which crystallized after drying with Na_2SO_4. The solvent was evaporated and the residue was chromatographed on SiO_2 (873 g). Elution with EtOAc/hexane (1 : 1) and removal of the solvent rendered 14.77 g (65%) of Fmoc-Asp(OPyBzh)-OBzl. TLC (system A): r_f 0.65; ESIMS: 689.1 (MH^+), 1376.5 (M_2H^+); ^{1}H-NMR ($CDCl_3$): δ 3.03–3.25 (2H, m, β-CH_2), 4.11–4.17 [1H, t, J = 7.1 Hz, H9 (Fmoc)], 4.31–4.41 [2H, m, O-CH_2 (Fmoc)], 4.68–4.70 (1H, m, α-CH), 5.00–5.14 [2H, m, O-CH_2 (Bzl)], 5.72–5.74 (1H, d, J = 8.4 Hz, NH), 7.25–7.30 [19H, m, arom. (Bzl, Bzh, Fmoc)], 7.34–7.38 [2H, d, J = 7.5 Hz, H3/5 (pyridine)], 7.51–7.53 [2H, d, J = 6.7 Hz, H1/8 (Fmoc)], 7.74–7.76 [2H, d, J = 7.6 Hz, H4/5 (Fmoc)], 8.49–8.50 [2H, d, J = 6.0 Hz, H2/6 (pyridine)].

Fmoc-Asp(OPyBzh)-OH (collidine salt)

Fmoc-Asp(OPyBzh)-OBzl (3.01 g, 4.37 mmol) was dissolved in EtOH (350 ml) at ambient temperature under nitrogen. Pd/C (0.5 g) was added and the ester was hydrogenated under atmospheric pressure. The conversion was monitored by TLC (system B). After 1.5 h, the catalyst was filtered off and the solvent removed *in vacuo*, leaving a foam which was redissolved in EtOAc (500 ml). Water (300 ml) and collidine (0.6 ml, 4.5 mmol) were added for extraction, followed by a wash with brine (200 ml). The crude material resulting after removal of the solvent was applied for chromatography on SiO_2 (206 g). The product was eluted with EtOAc/iPrOH (2 : 3). Evaporation of the solvent yielded 2.31 g (73%) of Fmoc-Asp(OPyBzh)-OH (collidine salt). The free acid used for NMR-analysis was obtained by treating a sample of the collidine salt with 0.05 M HCl after uptake in EtOAc followed by three aqueous washes, drying and evaporation of the solvent. TLC (system B): r_f 0.51; ESIMS: 599.2 (MH^+), 1196.8 (M_2H^+), 244.3 ($PyBzh^+$); ^{1}H-NMR ($CDCl_3$): δ 2.91–3.45 (2H, m, β-CH_2), 4.19–4.22 [1H, t, J = 7.1 Hz, H9 (Fmoc)], 4.27–4.40 [2H, m, O-CH_2 (Fmoc)], 4.67–4.69 (1H, m, α-H), 5.88–5.89 (1H, d, J = 8.1 Hz, NH), 7.24–7.40 [14H, m, arom. (Fmoc, PyBzh)], 7.55–7.57 [2H, d, J = 7.7 Hz, H3/5 (pyridine)], 7.59–7.61 [2H, d, J = 5.3 Hz, H1/8 (Fmoc)], 7.73–7.75 [2H, d, J = 8.0 Hz, H4/5 (Fmoc)], 8.49–8.50 [2H, d, J = 6.0 Hz, H2/6 (pyridine)].

Fmoc-Asp(OPhFl)-OAll

Fmoc-Asp-OAll (2.37 g, 6 mmol) was dissolved in dry DCM (15 ml). PhFl-Br (1.97 g, 6.1 mmol) and DIPEA (1.03 ml, 6 mmol) were added. The reaction was monitored by TLC (system D) and stopped after 19 h. After evaporation of the solvent, the residue was taken up in EtOAc/water (110 ml each) to remove DIPEA-HBr. Extraction with H_2O removed most of the unreacted Fmoc-Asp-OAll. After washing with brine (50 ml each), drying with Na_2SO_4 and evaporation of EtOAc, the crude Fmoc-Asp(OPhFl)-OAll was obtained as a white foam. Chromatography on SiO_2 eluting with hexane/EtOAc (4 : 1) afforded the desired product (3.03 g, 81%). TLC (system D): r_f 0.66; ESIMS: 658.1 (MNa^+), 674.1 (MK^+), 241.3 ($PhFl^+$); ^{1}H-NMR ($CDCl_3$): δ 2.92–3.20 (2H, m, β-CH_2), 4.17–4.20 [H, t, J = 7.2 Hz, H9 (Fmoc)], 4.27–4.41 [2H, m, O-CH_2 (Fmoc)], 4.46–4.55 [2H, m, O-CH_2 (All)], 4.63–4.67 (1H, m, α-CH), 5.14–5.17 [1H, m, CH=(All)], 5.20–5.24 [1H, m, CH=(All)], 5.71–5.77 (2H, m, =CH(All) and NH), 7.25–7.40 (15H, m, arom. (Fmoc, PhFl), 7.53–7.55 (2H, d, J = 7.3 Hz, arom.), 7.69–7.71 (2H, d, J = 7.5 Hz, arom.), 7.73–7.76 (2H, d, J = 7.6, arom.).

Fmoc-Asp(OPhFl)-OH (collidine salt)

Fmoc-Asp(OPhFl)-OAll (3.24 g, 5.1 mmol) was dissolved in dry DCM (50 ml) under nitrogen. $Pd(PPh_3)_4$ (0.12 g, 0.1 mmol), triphenylsilane (1.3 ml, 10 mmol) and collidine (0.7 ml, 5.28 mmol) were added consecutively. The homogeneous solution rapidly darkened, with the concomitant evolution of gas. TLC-monitoring (system B) indicated smooth allyl cleavage. The solvent was removed after 35 min and the amorphous residue was dissolved in EtOAc (150 ml). The resulting solution was washed with water and brine (2 × 80 ml each). The EtOAc solution yielded a dark foam upon evaporation. Redissolution in EtOAc and treatment with carbopal P1 removed the colour. Evaporation of the solvent yielded an off-white foam, which was applied to a column containing SiO_2 (124 g). Elution with EtOAc and EtOAc/iPrOH (1 : 1) afforded 2.17 g (61%) of Fmoc-Asp(OPhFl)-OH, collidine salt. The free acid used for NMR-analysis was obtained by treating a sample of the collidine salt in EtOAc with 2% aqueous citric acid followed by washes with water and brine, drying and evaporation. TLC

(system B): r_f 0.64; ESIMS (negative mode): 593.9 (M–H), 1189.7 (M_2–H); ^{1}H-NMR ($CDCl_3$): δ 2.92–3.17 (2H, m, β-CH_2, 4.16–4.19 [1H, t, J = 7.0 Hz, H9 (Fmoc)], 4.30–4.41 [2H, m, O-CH_2 (Fmoc)], 4.62–4.65 (1H, m, α-CH), 5.67–5.69 (1H, d, J = 8.6 Hz, NH), 7.16–7.39 (15H, m, arom.), 7.51–7.53 (2H, d, J = 7.6 Hz, arom.), 7.66–7.68 (2H, m, arom.), 7.73–7.75 (2H, d, J = 7.4 Hz, arom.).

Fmoc-Asp(O(3-methyloxetan-3-ylmethyl))-OBzl

Fmoc-Asp-OBzl (22.39 g, 50.2 mmol) was dissolved in dry THF (28 ml) followed by slow addition of oxalyl chloride (4.9 ml, 57.9 mmol). The evolution of gas was markedly enhanced when adding 2 drops of DMF. Evaporation after 4.5 h yielded a yellow solid, which was redissolved in a mixture of dry DCM (50 ml) and THF (10 ml). The solution of the acid chloride was added dropwise within 1 h to a mixture of 3-methyl-3-hydroxymethyl-oxetane (5.17 g, 50.6 mmol), dry DCM (50 ml) and pyridine (5 ml) at 0°C. Ester formation was monitored by TLC (system C). The ice-bath was removed after 2 h, and stirring was continued at room temperature overnight. EtOAc (750 ml) and water (250 ml) were added to extract the pyridinium salts. A further aqueous extraction was followed by treatment with 2% Na_2CO_3 (250 ml) to remove unreacted Fmoc-Asp-OBzl. After an additional aqueous wash, a brine extraction, treatment with Na_2SO_4 and evaporation of the solvent, 25.0 g (94%) of the desired ester was isolated. TLC: r_f 0.65 (system A), r_f 0.51 (system C).

Fmoc-Asp(OBO)-OBzl

After dissolution in dry DCM (100 ml), crude Fmoc-Asp[O(3-methyloxetan-3-ylmethyl)]-OBzl (24.87 g, max. 47.0 mmol) was subjected to ortho ester rearrangement [5]. Under Ar atmosphere, the solution was cooled with ice and BF_3OEt_2 (0.3 ml, 2.39 mmol) was added under vigorous stirring, then the ice-bath was removed. According to TLC (system C), the rearrangement proceeded rapidly, though a range of by-products could be detected. After 5 h, the reaction was quenched by addition of triethylamine (0.33 ml, 2.3 mmol). After evaporation, the crude product was purified by column chromatography on SiO_2 (1.1 kg, prewashed with collidine in EtOAc/hexane (1 : 2)). Upon removal of the solvent, Fmoc-Asp(OBO)-OBzl (13.0 g, 52%) crystallized. Mp: 136°–138°C. TLC: r_f 0.73 (system A), r_f 0.59 (system C); ESIMS: 552.2 (MNa^+), 1080.8 (M_2Na^+); ^{1}H-NMR: δ 0.78 [3H, s, CH_3 (OBO)], 2.19–2.41 (2H, m, β-CH_2), 3.83 [6H, s, O-CH_2 (OBO)], 4.25–4.28 [1H, t, J = 7.2 Hz, H9 (Fmoc)], 4.32–4.38 [2H, m, CH_2-O (Fmoc)], 4.54–4.56 (1H, m, α-CH_2), 5.16 [2H, s, O-CH_2 (Bzl)], 6.07–6.08 (1H, d, J = 9.3 Hz, NH), 7.25–7.41 [9H, m, arom. (Fmoc, Bzl)], 7.60–7.63 (2H, m, arom.), 7.75–7.77 (2H, d, J = 7.5, arom.).

Fmoc-Asp(OBO)-OH (collidine salt)

Fmoc-Asp(OBO)-OBzl (1.0 g, 1.88 mmol) was dissolved under an Ar atmosphere in dry THF (50 ml) and collidine (0.26 ml, 1.96 mmol) and Pd/C (60 mg) were added. Hydrogenation was carried out under atmospheric pressure. TLC (system B) indicated a smooth reaction. Hydrolysis of the OBO group was not observed. After 2.2 h the catalyst was removed followed by evaporation of the solvent. The product was obtained in quantitative yield (1.06 g) and it was used without further purification in SPPS. TLC (system B) r_f (orthoester) 0.53 (diol resulting from hydrolysis, r_f 0.28). ESIMS: 440.0 (MH^+), 462.1 (MNa^+), 458.2 (MH_3O^+ , produced by hydrolysis of OBO).

Assessment of the Acid-lability of OPyBzh and OPhFl Esters

To determine the stability of Fmoc-Asp(OPyBzh)-OBzl and Fmoc-Asp(OPhFl)-OAll in 1% TFA/DCM,

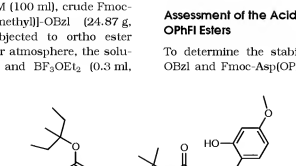


Figure 2 Structure of Fmoc-Asp(OMpe)-OH and Fmoc-Asp(OtBu)-HmbGly-OH.

the derivative (ca. 50 mg) was dissolved in DCM (1 ml). Then 2% TFA/DCM (1 ml) was added with vigorous stirring. The progression of the cleavage was followed by TLC (PyBzh: TLC system A, PhFl: TLC system D).

Solid Phase Synthesis of VKDGYI (I)

Solid phase synthesis was performed on the Wang resin and on Sasrin™ (see Results and Discussion). Fmoc was used for N^{α}-protection, *t*Bu and Boc were employed for side-chain protection of Tyr and Lys, respectively. The various β-carboxy protecting groups used for Asp are listed in Table 1. Piperidine/DMF (1 : 4) was the standard cleavage cocktail used for Fmoc removal up to the incorporation of the Fmoc-Asp derivative. Subsequent variations of Fmoc cleavage conditions are summarized in Table 2. In all cases, the resin was treated twice, for 5 and 10 min, with the base of choice. All couplings were performed using a three-fold excess of Fmoc amino acid derivative, TBTU

Table 1 Extent of Formation of Aspartimide and Other By-products during Syntheses of H-Val-Lys-Asp-Gly-Tyr-Ile-OH.

Protection	Desired product	Desired product(%)	Aspartimide (D and L) (%)	α-Piperidide (%)	β-Piperidide (%)	By-product[a] (%)
O*t*Bu	VKDGYI	91.1	2.3	1.5	nd	nd
OAll	VKD(OAll)GYI	nd	49.6	12.0	2.9	25.0
OPp	VKDGYI	80.7	9.0	1.1	0.3	1.5
OBzl	VKD(OBzl)GYI	1.5	63.6	12.3	2.0	14.2
OPyBzh	VKDGYI	1.0	55.8	12.2	2.6	14.8
OPhFl	VKDGYI	7.0	65.3	8.9	1.6	7.8
OBO	VKD(X)GYI[b]	85.3	6.1	0.9	nd	1.7
OMpe	VKDGYI	93.9	0.7	nd	nd	nd
O*t*Bu/Hmb	VKDGYI	94.0	nd	nd	nd	nd

Conditions of SPPS: see Materials and Methods. Fmoc removal was performed with piperidine/DMF (1 : 4), 5 and 10 min, at room temperature. Percentages of desired product and by-products were determined by RP-HPLC, as reported in Materials and Methods
[a] See Results and Discussion.
[b] X = $OCH_2C(CH_3)(CH_2OH)_2$.
nd = not detectable (<0.3%).

Table 2 Extent of Formation of Aspartimide and Other By-products during Syntheses of H-Val-Lys-Asp-Gly-Tyr-Ile-OH.

Protection	Desired product	Desired product(%)	Aspartimide (D and L) (%)	α-Piperidide (%)	β-Piperidide (%)	By-product[a] (%)
O*t*Bu	VKDGYI	91.1	2.3	1.5	nd	nd
O*t*Bu[b]	VKDGYI	81.9	7.2	1.2	nd	1.8
OMpe	VKDGYI	93.9	0.7	nd	nd	nd
OMpe[b]	VKDGYI	91.0	3.3	0.5	nd	0.7
O*t*Bu/Hmb	VKDGYI	94.0	nd	nd	nd	nd
O*t*Bu/Hmb[b]	VKDGYI	94.4	nd	nd	nd	nd

Conditions of SPPS: see Materials and Methods. Fmoc removal was performed with piperidine/DMF (1 : 4), 5 and 10 min, at room temperature or at 45 °C. Percentages of desired product and by-products were determined by RP-HPLC, as reported in Materials and Methods
[a] See Results and Discussion.
[b] Fmoc removal at 45 °C.
nd = not detectable (<0.3%).

and collidine in DMF. The coupling reaction was carried out for 1 h at ambient temperature. No additional collidine was applied for the coupling reaction in the cases of Fmoc-Asp derivatives that were already prepared as collidine salts. The pH was adjusted to pH 7 with collidine during the synthesis of the Asp(OPhFl)- or Asp(OBO)-containing peptides due to the acid-sensitivity of the side-chain protection. The syntheses were monitored by the Kaiser test and the 2,4,6-trinitrobenzenesulfonic acid test. Cleavage from the resin was performed with 95% aqueous TFA at room temperature for 1 h, followed by precipitation of the peptide with ice-cold *t*BuOMe. Alternatively, in the case of the Sasrin™, the protected peptides were obtained by repetitive short treatments (2 to 3 min) of the peptidyl resin with 1% TFA/DCM. The peptide-containing filtrates were neutralized immediately with pyridine, checked by TLC (system B) and evaporated. Conditions of analytical HPLC chromatography were: column: Baker-bond C_{18} 300Å; buffer system: 0.095 M phosphoric acid and 0.09 M triethylamine in water (pH 2.3), A: 5% CH_3CN, 95% buffer; B: 60% CH_3CN, 40% buffer; gradient: 5 to 35% B in 45 min; flow 1 ml/min; detection at 220 nm.

LC-MS identification of the most important products formed: 694.4 (MH^+, VKDGYI), 676.3 (MH^+, aspartimide formation), 761.3 (MH^+, piperidide formation), 577.3 (MH^+, further unidentified by-product, see Results and Discussion).

RESULTS AND DISCUSSION

An absolutely reliable protocol for the removal of the Fmoc group is a prerequisite for the SPPS of long peptides. Harsher cleavage methods which may ensure complete deblocking for larger peptides, enhance the risk of aspartimide formation and other base-induced side reactions. The extent of base-catalysed aspartimide formation varies considerably, depending on the type of base and the β-carboxy protecting group. However, Fmoc cleavage procedures known to be more efficient in Fmoc removal were also included in this study. In the case of high sensitivity towards bases, extensive cyclization followed by considerable amounts of further by-products resulting from the opening of the D/L-aspartimide ring were observed (see Figure 1). To be able to trace the following compounds, independent syntheses were performed: VKdGYI, VKd(GYI), VKD(GYI), VKD(piperidide)GYI and VKD(GYI)-piperidide. We did not synthesize the isomeric D-piperidides, although they may also be formed via the racemized aspartimide. With these derivatives in hand, HPLC conditions were optimized to obtain a satisfactory chromatographic separation of potential contaminants. A further by-product related to aspartimide formation could not be identified. MS suggests the loss of the *N*-terminal valine from the aspartimide-containing peptide.

Established Asp β-protecting Groups

The OtBu side-chain protection of Asp for the synthesis of **I** using 20% piperidine/DMF for Fmoc-cleavage at ambient temperature was the standard for the comparison against other derivatives. As indicated in Table 1, in the case of **I** by-products are easily generated. As expected, elevated temperatures, e.g. 45 degrees (see Table 2), promote the side reaction and also the stronger bases such as DBU or TMG give rise to a considerable amount of D/L-aspartimide and subsequent products. The latter results are listed in Table 3. Figure 7A shows a typical HPLC chromatogram if

Table 3 Fmoc Cleavage using Stronger Bases

Fmoc-Asp derivative	Desired product (%)	Aspartimide (D/L) (%)	α-Piperidide (%)	β-Piperidide (%)	By-product (%)
OtBu[a]	52.1	21.8	9.4	0.6	5.8
OtBu/Hmb[a]	94.1	nd	nd	nd	nd
OMpe[a]	83.0	7.8	1.9	nd	1.1
OtBu[b]	66.9	17.6	4.4	0.4	3.2

[a] DBU/piperidine/DMF (1:20:79).
[b] TMG/piperidine/DMF (2:20:78).
nd, not detectable (<0.3%).

1% DBU is applied for Fmoc removal after incorporation of the Asp residue applying OtBu protection. In addition, prolonged treatments with base, to mimic the conditions of the synthesis of long peptides, further increased the amount of D/L-aspartimide and related products. In particular, an extended contact with piperidine/DMF (an additional 3 h treatment of the peptide resin) indeed generated a remarkable amount of by-products (HPLC: VKDGYI 71.9%, L/D-aspartimide 11.4%, α-piperidide 6.3%).

Peptides containing an Asp β-allyl ester are rather sensitive towards base treatment during SPPS. Total conversion into aspartimide has been reported [6]. The SPPS of **I** employing Fmoc-Asp(OAll)-OH and 1% DBU/DMF for Fmoc cleavage [6] yielded aspartimide (HPLC: 84.8%) as the main product. In the presence of piperidine, piperidides and further by-products (see Table 1) were observed. In case of the derivative Fmoc-Asp(OBzl)-OH, a similar pattern of by-products appeared.

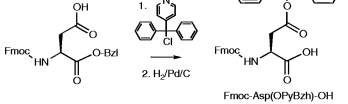
Aspartimide formation is impeded by bulky β-carboxy protecting groups and, in most cases, the β-OtBu-group provides sufficient steric hindrance. Therefore, the OPp protected Asp derivative [7] bearing the very acid-labile phenylisopropyl side-chain protecting group was included in our study. Surprisingly, this more bulky protecting group was somewhat more susceptible towards aspartimide formation in comparison to standard OtBu protection (see Table 1).

Synthesis and Application of new Asp Derivatives

Based on these observations, we decided to design even bulkier side-chain protecting groups and to test their potential to inhibit aspartimide formation. As a first example, the 4-pyridyl-diphenylmethyl ester (OPyBzh) protecting group was evaluated. This bulky moiety was expected to increase the base stability of **I** during synthesis while maintaining a reasonable sensitivity towards diluted TFA to facilitate postsynthetic cleavage. Thus, the derivative Fmoc-Asp(OPyBzh)-OH was synthesized starting from Fmoc-Asp-OBzl as outlined in Figure 3. An alternative synthesis starting from Z-Asp-OBzl failed due to the instability of the pyridylbenzhydryl ester under the conditions of hydrogenation. Consequently, prolonged hydrogenation of the corresponding Fmoc derivative had to be avoided. The first experiment was related to studies on the lability under acidic conditions. No significant cleavage of the PyBzh moiety was observed within a 5 h treatment with 1% TFA/DCM. By contrast, the PyBzh group was split off in 2% TFA/DCM within 30 min. Thus, the properties of this protecting group corresponded to our expectations and the Asp derivative was applied for the synthesis of **I**.

In the case of the PyBzh derivative, **I** could not be obtained by standard synthesis on Wang resin. Acidolytic cleavage yielded mostly aspartimide and the piperidides (see Table 1). Thus, the fully protected fragment was produced by synthesis on Sasrin™. Unexpectedly, the fully protected fragment Fmoc-Asp(OPyBzh)-Gly-Tyr(tBu)-Ile-OH instantaneously yielded the aspartimide upon treatment with only 2% piperidine/DMF. We reasoned that the pyridyl nitrogen may assist in aspartimide formation by a kind of neighbouring group effect.

Therefore, another trityl analogue for β-carboxy protection of Asp was synthesized, the 9-phenylfluorenyl ester(OPhFl). The PhFl ester has been recently described [9], albeit the corresponding Fmoc-Asp



Fmoc-Asp(OPyBzh)-OH

Figure 3 Synthesis of Fmoc-Asp(OPyBzh)-OH.

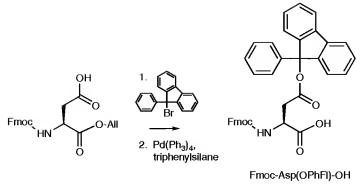


Figure 4 Synthesis of Fmoc-Asp(OPhFl)-OH.

derivative has not been produced so far. Fmoc-Asp(OPhFl)-OH was obtained starting from Fmoc-Asp-OAll as indicated in Figure 4. In our hands, several hours were required to completely cleave the PhFl group with 0.5% TFA/DCM and also removal by hydrogenation could be easily achieved. Since the conditions for removal agreed with the reported properties of the PhFl group, this derivative was applied for synthesis of the test peptide. Surprisingly, synthesis of **I** on Wang resin using the derivative Fmoc-Asp(OPhFl)-OH (collidine salt) afforded only the aspartimide and the piperidides (see Table 1). For studying the behaviour of the OPhFl-group in more detail another synthesis of **I** was started on Sasrin™. Mild acidolysis of a sample taken after the coupling of Fmoc-Asp(OPhFl)-OH yielded the expected intermediate Fmoc-Asp-Gly-Tyr(*t*Bu)-Ile-OH. Cleavage of a sample taken after piperidine treatment afforded a mixture of the desired product H-Asp-Gly-Tyr(*t*Bu)-Ile-OH, aspartimide and piperidide as detected by MS. Further cleavages following stepwise elongation showed that the fraction of the desired product rapidly decreased. According to HPLC analysis, a mixture containing D/L-aspartimide (63.0%), α-piperidide (12.8%), β-piperidide (2.3%), and only a trace of the desired product **I** was obtained after final cleavage. There is evidence that only a few piperidine treatments are sufficient to significantly decrease the quality of an Asp-Gly containing peptide via concomitant aspartimide formation. This finding is more difficult to interpret, but it is in accordance with the observation of Kunz *et al.* [9] on the instability of the PhFl group under basic conditions. In the latter publication, the formation of pyroglutamic acid from *N*-terminal Glu(OPhFl) during prolonged treatment with morpholine was described.

The results on trityl protection are somehow in line with the observation of increased amounts of side products observed in case of OFp protection. Obviously, aromatic residues cannot sufficiently suppress the attack of the amide nitrogen and, furthermore, due to the enhanced lability, they promote the leaving group character of these protecting groups which result in a higher propensity to form the aspartimide. Due to these disappointing results, trityl-type protecting groups were not further investigated.

The transformation of the β-carboxy group was thought to be an alternative approach to ensure complete suppression of aspartimide formation. We anticipated that the bicyclic OBO orthoester [10] would be optimal Asp β-carboxy protection during Fmoc/*t*Bu assisted SPPS. Fmoc-Asp(OBO)-OH (isolated as the collidine salt) was difficult to synthesize (see Figure 5), but with the derivative in hand, a synthesis of **I** was performed. Following TFA treatment, the desired product H-Val-Lys-Asp[O-2,2-di(hydroxymethyl)propyl]-Gly-Tyr-Ile-OH was obtained. However, a small amount of the α-piperidide was detected (see Table 1). This finding can only be explained assuming slow hydrolysis of the orthoester moiety during SPPS (see Figure 6), aspartimide formation from the resulting Asp β-[2,2-di(hydroxymethyl)propyl] ester, and subsequent cleavage by piperidine. Furthermore, large quantities of aspartimide are formed during the second stage of OBO removal, which involves the saponification of the 2,2-di(hydroxymethyl)propyl ester (see Figure 6). The mild conditions, as developed by Ramage *et al.* [11] for the saponification of the 2,2-di(hydroxymethyl)-2-nitro-ethoxycarbonyl group, failed in our case. In summary, conditions for basic hydrolysis could not be adjusted to avoid the known side reactions.

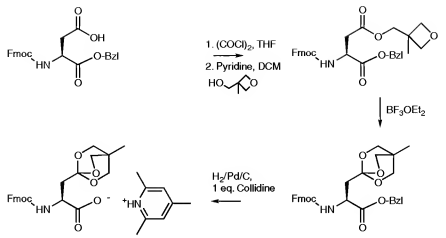


Figure 5 Synthesis of Fmoc-Asp(OBO)-OH.

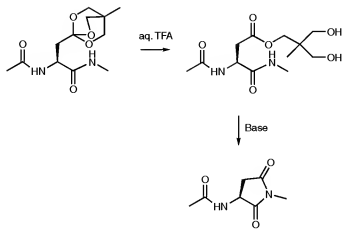


Figure 6 Removal of the OBO protecting group.

Hence, standard OtBu protection was reconsidered and possibilities for a systematic improvement were sought. Firstly, the synthesis of **I** incorporating an Fmoc-Asp derivative having the more bulky 3-methylpent-3-yl ester (OMpe) [8] (see Figure 2) attached to the β-carboxy functionality was studied. Especially the results obtained on synthesis of **I** under harsher conditions (1% DBU), clearly showed an improvement compared with OtBu protection (see Table 3 and Figure 7B). These results were corroborated by the finding that elevated temperatures (45 degrees) have a more pronounced effect on aspartimide formation in the case of standard OtBu protection (see Table 2).

Next, the effect of backbone protection in our test system should be established clearly. Backbone protection (Hmb) was reported in the literature [2,12] and the alkylation of the Asp–Xaa amide bonds was thought to be an efficient method for preventing aspartimide formation. The dipeptide Fmoc-Asp(OtBu)-HmbGly-OH was produced and applied for the synthesis of **I**. The derivative coupled smoothly to the resin after activation with the more efficient coupling reagent TATU. During acidolysis, the Hmb group was readily removed. Even under harsher conditions, e.g. stronger base or elevated temperature, no aspartimide-related by-products could be detected (see Tables 1–3, Figure 7C). Thus, the combination of Asp(OtBu) side chain protection with Hmb backbone protection on the carboxy side is an efficient combination to completely suppress aspartimide formation. However, it remains

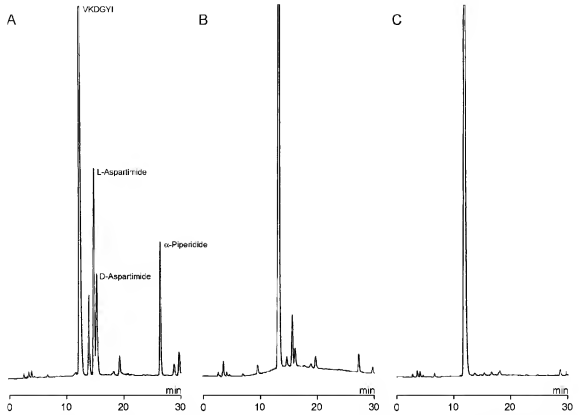


Figure 7 Analytical HPLC-profiles of crude products obtained after syntheses of peptide **I** using DBU/piperidine/DMF (1:20:79) for Fmoc cleavage. A: Synthesis performed with Fmoc-Asp(OtBu)-OH, B: Synthesis performed with Fmoc-Asp(OMpe)-OH, C: Synthesis performed with Fmoc-Asp(OtBu)-HmbGly-OH.

to be determined whether the accessibility of the dipeptide building block and its racemization-free incorporation can be realised for amino acids other than Gly.

CONCLUSION

This investigation clearly showed that the introduction of backbone protection reliably prevents aspartimide formation in the case of the sensitive Asp-Gly sequence. Among the numerous Asp side-chain protecting groups evaluated in the course of this study, only the slightly more bulky OMpe-group turned out to be superior to the standard *O*tBu protection with respect to suppression of aspartimide-related side reactions. Alternative protecting groups which might have been expected to prevent aspartimide formation, such as trityl ester and orthoester derivatives, turned out to rather promote this undesired reaction. In continuation of this work, we plan to include other amino acids in place of Gly, and this will be the subject of future reports.

REFERENCES

1. Lauer JL, Fields CG, Fields GB. Sequence dependence of aspartimide formation during 9-fluorenylmethoxycarbonyl solid-phase peptide synthesis. *Lett. Peptide Sci.* 1994; **1**: 197–205.
2. Wade JD, Mathieu MN, Macris M, Tregear GW. Base-induced side reactions in Fmoc-solid phase peptide synthesis: Minimization by use of piperazine as N^{α}-deprotection reagent. *Lett. Peptide Sci.* 2000; **7**: 107–112.
3. Nicolás E, Pedroso E, Giralt E. Formation of aspartimide peptides in Asp-Gly sequences. *Tetrahedron Lett.* 1989; **30**: 497–500.
4. Coyle S, Hallett A, Munns MS, Young GT. Amino-acids and peptides, Part 45. The protection of the thiol function of cysteine and the imidazole-*N* of histidine

by the diphenyl-4-pyridylmethyl group. *J.Chem.Soc. Perkin Trans I*, 1981; 522–528.
5. Corey EJ, Raju N. A new general synthetic route to bridged carboxylic ortho esters. *Tetrahedron Lett.* 1983; **24**: 5571–5574.
6. Vigil-Cruz SC, Aldrich JV. Unexpected aspartimide formation during coupling reactions using Asp(OAl) in solid-phase peptide synthesis. *Lett. Peptide Sci.* 1999; **6**: 71–75.
7. Yue C, Thierry J, Potier P. 2-Phenyl isopropyl esters as carboxyl terminus protecting groups in the fast synthesis of peptide fragments. *Tetrahedron Lett.* 1993; **34**: 323–326.
8. Karlström A, Undén A. A new protecting group for aspartic acid that minimizes piperidine-catalyzed aspartimide formation in Fmoc solid phase peptide synthesis. *Tetrahedron Lett.* 1996; **37**: 4243–4246.
9. Löhr B, Orlich S, Kunz H. Modified trityl ester protecting groups in peptide synthesis. *Synlett* 1999; 1136–1138.
10. Blaskovich MA, Evindar G, Rose NGW, Wilkinson S, Lao Y, Lajoie GA. Stereoselective synthesis of *threo* and *erythro* β-hydroxy- and β-disubstituted β-hydroxy α-amino acids. *J. Org. Chem.* 1998; **63**: 3631–3646.
11. Ramage R, Biggin GW, Brown AR, Comer A, Davison A, Draffian L, Jiang L, Morton G, Robertson N, Shaw KT, Tennant G, Urquhart K, Wilken J. Methodology for chemical synthesis of proteins. In *Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Libraries*. Mayflower Scientific: Birmingham 1996; 1–10.
12. Offer J, Quibell M, Johnson T. On-resin solid-phase synthesis of asparagine *N*-linked glycopeptides: use of *N*-(2-acetoxy-4-methoxybenzyl) (AcHmb) aspartyl amide bond protection to prevent unwanted aspartimide formation. *J. Chem. Soc. Perkin Trans. I* 1996; 175–182.

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Microwave-enhanced liquid-phase synthesis of thiohydantoins and thioxotetrahydropyrimidinones

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Summary

An efficient, microwave-assisted method for the liquid-phase combinatorial synthesis of 3,5-disubstituted thiohydantoins and 3,5-disubstituted 2-thioxotetrahydropyrimidin-4-ones has been developed. In synthesizing thiohydantoins, Fmoc-protected amino acids were coupled with polymer support and then deprotected to give primary amines. While in synthesizing thioxotetrahydropyrimidinones, 3-chloropropionyl chloride was immobilized to the support and subsequently reacted with various amines to form secondary amines. The PEG bound primary/secondary amines then were incorporated with various isothiocyanates to give thiourea intermediates and concomitant cyclization/cleavage steps occurred under mild basic condition. The desired products were then liberated from the soluble matrix in good yield and purity. All reactions described here were performed under microwave irradiation.

Introduction

With more and more therapeutic targets emerging from chemical genomics research, there is an urgent need to find efficient ways to synthesize biologically active molecules. Combinatorial chemistry provides a fast access to large quantities of structurally diverse compound collections to fuel the chemical genetics. Limitations in the efficiency of classical chemical synthesis resulting from tedious work-up and purification after each reaction step can be overcome by the solid phase synthesis due to advantages like easy and fast purification to separate excess reagents and side products from the desired compounds [1]. It is expected that solid phase synthesis, using known solution-phase reaction conditions, will be useful for the development of efficient methodologies to find novel therapeutics [2]. However, solid-phase chemistry suffers from various problems such as the heterogeneous nature of reaction conditions, reduced rates of reactions, solvation of the bound species and mass transport of reagents. We have been interested in employing liquid-phase combinatorial technology as a means of efficiently constructing diverse multifunctional libraries [3]. This strategy enables standard solution-based chemistry to be utilized and the product purification is just like that of solid phase reactions. Furthermore, monitoring the progress of reactions on the support is significantly simplified by using conventional analytical methods such as NMR, IR, HPLC and TLC [4].

Recently combinatorial organic synthesis has focused on the generation of non-peptide small molecules with potential therapeutical value. Compounds with the hydantoin structural motif have been identified to display a wide range of biological activities [5–11]. Hydantoins have been receiving the attention of researchers, and some of them have been approved by the U.S. Food and Drug Administration (FDA). For example, phenytoin has many uses, such as antiarrhythmic, anticonvulsant, antineuralgic, trigeminal neuralgia and

skeletal muscle relaxant. Sulfahydantoin has been studied with respect to inhibition of serine proteases. The glucopyranosylidene-spiro-thiohydantoin is reported as an efficient inhibitor of muscle and liver glycogen phosphorylases. For disubstituted-2-thioxotetrahydropyrimidin-4-ones, analogs of hydantoins, it was reported that the six-membered ring provided a better spatial configuration of the substituent to exert its hypoglycemic activity. The basic scaffold of hydantoin is therefore used to design various analogs, which are shown to have antiarrhythmic, anticonvulsant and antineuralgic activities. (Figure 1)

In order to quickly generate compound libraries of increasing molecular diversity, it would be favorable to develop methods that combine the expediency of microwave energy with the flexibility of soluble polymer supported combinatorial chemistry. The practicality of microwave irradiation in chemical reaction enhancement has been recognized for increasing reaction rates and formation of cleaner products [12]. It is clear that the synergistic application of microwave technology to rapidly synthesize biologically significant molecules on the support [13] would be a great advantage for accelerated library generation and as a useful tool for a drug-discovery program [14].

The synthesis of hydantoin is similar to the well-known Edman degradation, the cyclic cleavage of peptides based on the reaction of isothiocyanate with the free amino of the N-terminal residue such that amino acids are removed. Although a number of strategies for the synthesis of hydantoin analogs libraries have been reported including solid phase synthesis [15]–[29] and solution synthesis [30]–[31], application of microwave technology to facilitate multi-step thiohydantoins and thioxotetrahydropyrimidinone synthesis on soluble support has not been demonstrated. We adapted herein a hybrid strategy using both combinatorial and microwave approaches from readily available building blocks to the expeditious synthesis of thiohydantoin/thioxotetrahydropyrimidinone derivatives.

Results and discussion

The general synthetic route to thiohydantoins is described in Scheme 1. The soluble polymer support (HO-PEG-OH, MW ~ 6000) dissolved in methylene dichloride was coupled with Fmoc-protected amino acids under DCC/DMAP activation conditions in the microwave cavity for 14 min. Reaction mixtures were purified through a simple precipitation, filtration and ether washing to remove un-reacted reagents and side products. After drying, compound **1** was analyzed by routine ^{1}H-NMR to check the loading percentages on PEG support (Figure 2). In Figure 2 (**A** → **E**), we demonstrated how the conventional ^{1}H NMR spectroscopy was used to monitor the preparation of compound **4a**. The proton NMR spectrum of polymer support was given as spectrum **A**. Compound **1a** was obtained after OH-PEG-OH was coupled with Fmoc-protected valine and showed three sets of characteristic chemical shifts in spectrum **B**: (1) δ 0.8 ~ 1.0 (dd) represented the two methyl groups of valine, (2) δ 4.2 ~ 4.4 (m) represented $H_{c'}$ of PEG-OCH_2CH_2-OCO- as well as H_c, and (3) δ 7.3 ~ 7.5 (m) represented the aromatic protons of Fmoc protective group. Spectrum **C** was recorded to confirm that compound **1a** was completely deprotected to give compound **2a**; the aromatic proton signals of the Fmoc group, δ 7.3 ~ 7.5 (m), disappeared. In spectrum **D**, the aromatic protons H_d of the phenyl group on thiourea intermediate **3a** were shown at δ 7.3 ~ 7.5. Finally, the desired thiohydantoin **4a** was released from the support and its spectrum was recorded as spectrum **E**. With this non-destructive monitoring method and experiences, each intermediate could be investigated thoroughly using standard ^{1}H NMR spectroscopy.

The same work-up precipitation and proton NMR analysis protocol have been followed at every step of the present reaction sequence. Deprotection of the Fmoc group from compound **1** was attempted with microwave irradiation, but it was found that the amino acids were cleaved from the support. Instead, deprotection of compound **1** was performed with 10% piperidine in dichloromethane at room temperature for one hour. Various isothiocyanates were then incorporated through 150 W microwave irradiation for 7 min in dichloromethane to give thiourea intermediate **3**. The cyclization/traceless cleavage step was completed under mild basic condition (K_2CO_3) with 150 W microwave flash heating for 7 min [32]. Upon completion of the reaction, the polymer support was removed from the homogeneous solution by precipitation and filtration to provide the corresponding crude products **4** in 87–99% yield calculated on the basis of the initial loading to the support. The desired compounds were obtained with 81–99% purity as assessed by HPLC (Table 1).

We felt it was worth synthesizing analogues where the ring size is expanded by one unit. This ring expansion approach is much the same as changing

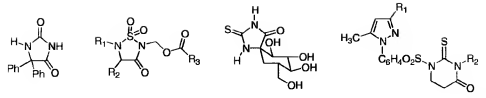
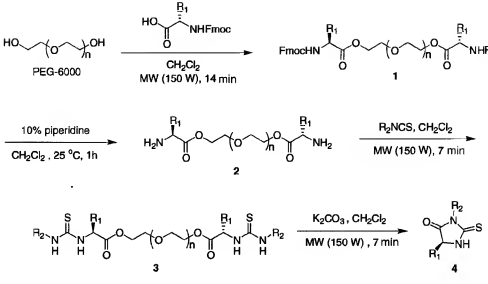


Figure 1. Examples of medicinally interesting hydantoin analogs.



Scheme 1. Synthesis of 3,5-disubstituted thiohydantoins.

the substitution pattern of a template that puts the binding groups in various patterns. To increase the molecular diversity based on the thiohydantoin motif, the synthesis of thioxotetrapyrimidinones was carried out as shown in Scheme 2. The soluble polymer support (HO-PEG-OH, MW $\sim$ 6000) dissolved in methylene chloride was reacted with 3-chloropropionyl chloride in the microwave cavity for 4 min. Upon completion of the reaction, the compound mixtures were purified by precipitation and thoroughly washed with solvents to remove un-reacted reagents and side products. Conventional ^{1}H NMR monitoring was performed in each step (Figure 3). In Figure 3 (**A** $\rightarrow$ **I**), we took the same strategy to check on the preparation of 1-butyl-3-phenyl-2-thioxo-tetrahydropyrimidine-4-one (**8a**) by conventional ^{1}H NMR spectroscopy. Spectrum **A** showed the chemical shift of poly (ethylene glycol) at δ 3.95 $\sim$ 3.30. After reaction with 3-chloropropionyl chloride, the polymer immobilized chloropropionyl ester **5a** was recorded as spectrum **F**; two sets of characteristic signals appeared: δ 4.27 (t) and 2.18 (t) represented H_a and H_b of HO-(CH$_2$CH$_2$O)$_n$COCH$_2$CH$_2$Cl, respectively. In spectrum **G**, $H_{d,e,f}$ of n-butyl group on the support of compound **6a** were observed at δ 1.5 $\sim$ 1.6 (m), δ 1.2 $\sim$ 1.4 (m) and δ 0.9 (t), respectively. In spectrum **H**, the aromatic protons H_g of phenyl group on thiourea intermediate **7a** were shown at δ 7.3 $\sim$ 7.8. The cyclo-cleaved poduct **8a** was then recorded as spectrum **I**. Again, through spectrum **A** $\rightarrow$ **I**, the reaction progress on the immobilized intermediates was monitored directly with conventional ^{1}H NMR spectroscopy without cleaving the products from the polymer support.

In the transamination step, many type of solvents, such as dichloromethane, chloroform, dichloroethane

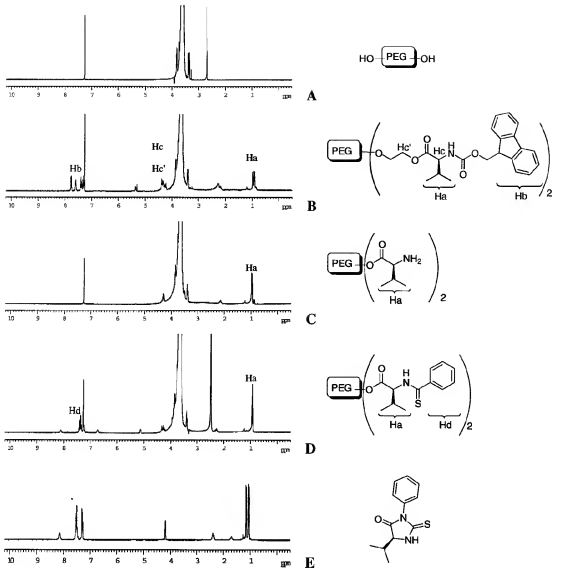


Figure 2. ^{1}H NMR monitoring of a stepwise thiohydantoin (**4a**) formation.

and tetrahydrofuran, have been used in this reaction. However, it took about 48 h to accomplish acceptable conversion at ambient temperature. Under refluxing condition, nucleophilic substitution of the polymer immobilized chloropropionyl ester **5** with several primary amines still required seven hours to reach completion. The same reaction was complete within 15 min under 200 W microwave heating. Meanwhile, in the ^{1}H NMR monitoring spectra, some trial double bond signals at δ 5.6–6.5 were observed early in the reaction and then disappeared when the conversion was complete. We propose that the α,β-unsaturated ester intermediate **6a** is formed first, followed by 1,4-conjugate addition of the primary amine to the double bond (Scheme 2). This proposal was confirmed by coupling acryloyl chloride with PEG to form the same intermediate **6a**. The ^{1}H NMR spectrum of **6a** was compared with the monitoring spectrum (Figure 3).

Table 1. Representative products and results of 3,5-disubstituted thiohydantoins **4**

Entry	R_1	R_2-NCS	LRMS	Crude yield[a]	Crude purity[b]
4a			234	97 %	99 %
4b			248	90 %	87 %
4c			214	88 %	87 %
4d			252	94 %	96 %
4e			279	99 %	99 %
4f	H		172	90 %	81 %
4g	H		210	95 %	96 %
4h			296	97 %	97 %
4i			327	94 %	97 %
4j			228	96 %	91 %
4k			262	90 %	99 %
4l			266	97 %	99 %
4m			248	98 %	97 %
4n			293	87 %	95 %

a: Determined based on weight of crude sample (%).
b: Purity determined by HPLC analysis (UV detection at λ= 254nm) of crude product (%).
Hypersil silica column, 250*4.6 mm, 5u

Following ether washing and drying of PEG bound secondary amines **6**, various isocyanates (2.2 eq) were incorporated through 90 W microwave irradiation for 10 min to give thiourea intermediates **7**. When larger excess of isothiocyanates were used to drive reactions to completion, final product purification was complicated and should be prevented. The cyclization/traceless cleavage [30] step was finished under mild basic condition (K_2CO_3) with 150 W microwave flash heating for 7 min. The representative library of 5-

disubstituted-2-thioxotetrahydropyrimidin-4-ones and analytical data are listed in Table 2.

The major advantage of cyclo/release strategy is the fact that only the desired compound is released into the solution. After completion of the reaction, the polymer support was removed from the homogeneous solution by precipitation and filtration to provide the corresponding crude products **8** in 80–98% yield calculated on the basis of the initial loading to the support. The desired compounds were obtained with 80–99% purity as assessed by HPLC (Table 2).

The structural characterization of cleaved libraries demonstrates the success of the major transformations described in Scheme 1 and 2. Products from the validated libraries were characterized by mass spectrometry and proton NMR confirming that in each reaction the major compound has a molecular weight corresponding to the appropriate product.

Conclusions

In summary, we have successfully combined the advantages of microwave technology with liquid phase combinatorial chemistry to facilitate thiohydantoins and thioxotetrapyrimidinones synthesis and to rapid reaction optimization. Purification steps are minimized, analytical methods are significantly simplified and a very defined product is yielded. Microwave irradiation is a powerful tool for accelerating reaction rates dramatically. Compared to conventional thermal heating, microwave irradiation decreased the reaction time on the support from several days to several minutes. It is also worth to note that the polymer supported intermediates and polymer support itself remain stable under microwave exposure. The coupling of microwave technology with liquid- phase combinatorial synthesis constitutes a novel and attractive avenue for the rapid generation of structurally diverse libraries.

Experimental section

General

Dichloromethane was distilled from calcium hydride before use. All reactions were performed under an inert atmosphere with unpurified reagents and dry solvents. Analytical thin-layer chromatography (TLC) was performed using 0.25 mm silica gel coated Kieselgel 60 F_{254} plates. Flash chromatography was performed using the indicated solvent and silica gel 60 (Merck, 230–400 mesh). Microwave flash heating was performed in CEM Discover equipment. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker DX-300 spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. High-resolution mass spectra (HRMS) were recorded on a JEOL TMS-HX 110 mass spectrometer. Normal phase HPLC was performed on a Shimadzu LC-10AT series machine with a Hypersil (250 × 4.6 mm) analytical column. PEG was purchased from SHOWA. Fmoc-protected amino acids were purchased from Advanced ChemTech.

General procedure for the synthesis of thiohydantoins (**4a–4n**)

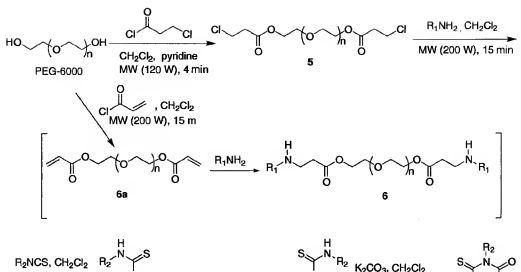
Polymer support (1 g, 0.17 mmol) dissolved in 10 mL of dichloromethanol was coupled with Fmoc-protected amino acids (0.50 mmol) under DCC/DMAP (0.50 mmol 0.009 mmol^{-1}) activation condition and heated in the microwave cavity for 14 min. To the reaction mixture was added ether in order to precipitate PEG-supported intermediates, which was filtered and washed with ether to remove un-reacted reagents and side products. Deprotection of compound **1** (500 mg) was performed with 10% piperidine (1 mL) in 9 mL of dichloromethane at 25 ° for one hour to obtain polymer bound diamine **2**. A mixture of polymer bound diamine **2** (500 mg) and phenyl isothiocyanate (3.0 equiv.) in 5 mL of CH_2Cl_2 was irradiated in the microwave cavity with an output of 150 watt for 7 min. Upon completion of the reaction, ether (20 mL) was added to the reaction mixture to precipitate the PEG-bound thiourea compound **3**. The precipitate was then collected on a sintered glass funnel and thoroughly washed with diethyl ether (20 ml × 3) following filtration. Finally, the desired cyclized thiohydantoin was released from the support by heating in the microwave cavity with an output at 150 watt for 7 min with K_2CO_3 (3 equiv.) in dichloromethane. The combined filtrate was dried to offer the corresponding crude product.

5-Isopropyl-3-phenyl-2-thioxo-imidazolidin-4-one (**4a**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (bs, –NH), 7.55 ~ 7.43 (m, 3H), 7.32 ~ 7.26 (m, 2H), 4.18 (d, J = 3.7 Hz, 1H), 2.44 ~ 2.31 (m, 1H), 1.14 (d, J = 6.9 Hz, 3H), 1.04 (d, J = 6.7Hz, 3H); ^{13}C NMR (75 MHz,

Table 2. Representative products and results of thioxotetrahydropyrimidinones **8**

Entry	R_1NH_2	R_2-NCS	LRMS	Crude yield[a]	Purity[b]
8a			262	91 %	95 %
8b			307	98 %	65 %
8c			312	95 %	94 %
8d			226	90 %	99 %
8e			266	92 %	87 %
8f			293	80 %	84 %
8g			300	96 %	80 %
8h			300	95 %	97 %
8i			316	96 %	84 %
8j			316	95 %	83 %
8k			297	91 %	82 %
8l			261	87 %	89 %
8m			297	86 %	98 %
8n			311	90 %	92 %

[a] Determined based on weight of crude sample (%).
[b] Purity determined by HPLC analysis (UV detection at λ = 254 nm) of crude product (%). Hypersil silica column, 250*4.6 mm, 5u



Scheme 2. Synthesis of 3,5-disubstituted-2-thioxotetrahydropyrimidin-4-ones **8**.

$CDCl_3$): δ 184.3, 173.0, 132.6, 129.4, 129.3, 128.3, 65.0, 31.2, 18.8, 16.2; IR (cm^{-1}, neat): 2965, 2911, 1757, 1591, 1516, 1407, 1348; Mass spectrum (EI) m/z 234 (M^+). Exact mass calcd for $C_{12}H_{14}N_2OS$: m/z 234.0827. Found 234.0828.

5-isopropyl-2-thioxo-3-m-tolyl-imidazolidin-4-one (**4b**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.43 (bs, –NH), 7.43 ~ 7.37 (m, 1 H), 7.29 ~ 7.26 (m, 1 H), 7.08 (m, 2 H), 4.18 ~ 4.16 (m, 1 H), 2.42 (s, 3 H), 2.39 ~ 2.30 (m, 1 H), 1.13 (d, J = 6.9 Hz, 3 H), 1.04 (d, J = 6.7 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.4, 173.2, 139.4, 132.5, 130.3, 129.1, 128.8, 125.4, 65.1, 31.2, 21.4, 19.0, 16.2; IR (cm^{-1}, neat): 3172, 2965, 2911, 1755, 1614, 1587, 1512, 1461, 1402, 1345; Mass spectrum (EI) m/z 248 (M^+) Exact mass calcd for $C_{13}H_{16}N_2OS$: m/z 248.0983. Found 248.0979.

3-Butyl-5-isopropyl-2-thioxo-imidazolidin-4-one (**4c**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.19 (bs, –NH), 4.00 ~ 3.96 (m, 1H), 3.73 ~ 3.81 (m, 2H), 2.34 ~ 2.21 (m, 1H), 1.68 ~ 1.57 (m, 2H), 1.36 ~ 1.24 (m, 2H), 1.10 ~ 1.05 (m, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.5, 173.8, 64.6, 41.0, 30.8, 29.7, 20.0, 18.9, 16.2, 13.7; IR (cm^{-1}, neat): 2961, 2929, 1747, 1512, 1434, 1407, 1351; Mass spectrum (EI) m/z 214 (M^+). Exact mass calcd for $C_{10}H_{18}N_2OS$: m/z 214.1140. Found 214.1139.

3-(4-Fluoro-phenyl)-5-isopropyl-2-thioxo-imidazolidin-4-one (**4d**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.26 (bs, –NH), 7.31 ~ 7.24 (m, 2H), 7.23 ~ 7.16 (m, 2H), 4.18 (d, J = 3.0 Hz, 1H), 2.44 ~ 2.33 (m, 1H), 1.14 (d, J = 7.0 Hz, 3H), 1.04 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.1, 173.0, 162.7 (d, $^1J_{CF}$ = 248 Hz), 130.2 (d, $^3J_{CF}$ = 8 Hz), 128.4 (d, $^4J_{CF}$ = 3 Hz), 116.3 (d, $^2J_{CF}$ = 23 Hz), 65.1, 31.2, 18.8, 16.2; IR (cm^{-1}, neat): 3172, 2965, 2920, 1759, 1512, 1461, 1411, 1345; Mass spectrum (EI) m/z 252 (M^+). Exact mass calcd for $C_{12}H_{13}FN_2OS$: m/z 252.0733. Found 252.0729.

5-Isopropyl-3-(4-nitro-phenyl)-2-thioxo-imidazolidin-4-one (**4e**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.41 (bs, –NH), 8.36 (dd, J = 7.0, 1.9 Hz, 2H), 7.57 (dd, J = 7.0, 1.9 Hz, 2H), 4.24 (d, J = 3.7 Hz, 1H), 2.43 ~ 2.36 (m, 1H), 1.15 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.8, 172.3, 147.7, 138.0, 129.3, 124.4, 65.2, 31.3, 18.8, 16.4; IR (cm^{-1}, neat): 2956, 2911, 1758, 1614, 1596, 1523, 1348; Mass spectrum (EI) m/z 279 (M^+). Exact

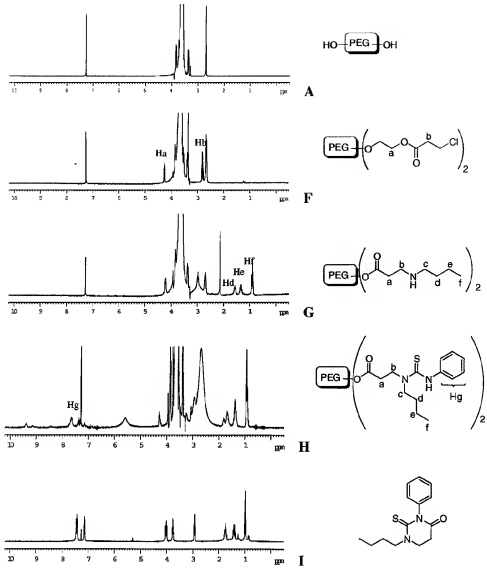


Figure 3. ^{1}H NMR monitoring of a stepwise thioxotetrapyrimidinone (**8a**) formation.

mass calcd for $C_{12}H_{13}N_3O_3S$: m/z 279.0678. Found 279.0680.

3-Butyl-2-thioxo-imidazolidin-4-one (**4f**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10 (bs, –NH), 4.06 (d, J = 0.9 Hz, 2H), 3.81 (t, J = 7.5 Hz, 2H), 1.70 ~ 1.60 (m, 4H), 1.27 ~ 1.23 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.3, 171.5, 48.3, 41.3, 29.6, 20.0, 13.7; IR (cm^{-1}, neat): 2956, 2911, 1713, 1506, 1434, 1345; Mass spectrum (EI) m/z 172 (M^+). Exact mass calcd for $C_7H_{12}N_2OS$: m/z 172.0670.

3-(4-Fluoro-phenyl)-2-thioxo-imidazolidin-4-one (**4g**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 ~ 7.28 (m, 2H + –NH), 7.24 ~ 7.17 (m, 2H), 4.30 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 170.7, 162.8 (d, $^1J_{CF}$ = 248 Hz), 130.2 (d, $^3J_{CF}$ = 9 Hz), 128.4, 116.4 (d, $^2J_{CF}$ = 23 Hz), 48.9; IR (cm^{-1}, neat): 2911, 1731, 1506;

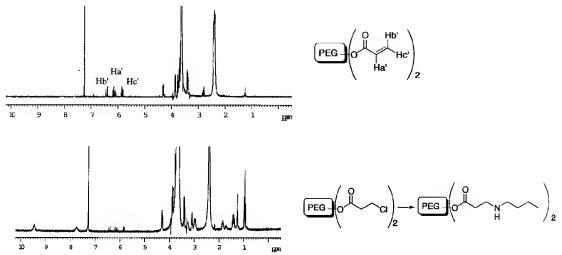


Figure 4. ^{1}H NMR spectrum of compound **6a** and reaction monitoring transamination.

Mass spectrum (EI) m/z 210 (M^+). Exact mass calcd for $C_9H_7FN_2OS$: m/z 210.0263. Found 210.0261.

5-Benzyl-2-thioxo-3-m-tolyl-imidazolidin-4-one (**4h**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 ~ 7.33 (m, 4H), 7.33 ~ 7.23 (m, 3H), 7.23 (bs, –NH), 6.86 ~ 6.90 (m, 2H), 4.53 (dd, J = 8.1, 3.6 Hz, 1H), 3.38 (dd, J = 14.1, 3.6 Hz, 1H), 3.08 (dd, J = 14.1, 8.1 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.8, 172.7, 139.3, 134.3, 132.4, 130.3, 129.4, 129.2, 129.0, 128.7, 127.9, 125.2, 60.9, 37.8, 21.4; IR (cm^{-1}, neat): 2956, 1749, 1650, 1605, 1497; Mass spectrum (EI) m/z 296 (M^+). Exact mass calcd for $C_{17}H_{16}N_2OS$: m/z 296.0983. Found 296.0973.

5-Benzyl-3-(4-nitro-phenyl)-2-thioxo-imidazolidin-4-one (**4i**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (dd, J = 9.0, 2.2 Hz, 2H), 7.40 ~ 7.35 (m, 4H), 7.32 (d, J = 2.1 Hz, 1H), 7.31 ~ 7.29 (m, 1H), 7.28 ~ 7.25 (m, 1H), 4.60 (dd, J = 7.5, 3.5 Hz, 1H), 3.39 (dd, J = 13.9, 3.4 Hz, 1H), 3.13 (dd, J = 13.9, 7.6 Hz, 1H), –NH proton signal was not observed.; ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.2, 171.8, 147.7, 137.8, 133.8, 129.4, 129.2, 128.1, 124.3, 61.0, 37.8; IR (cm^{-1}, neat): 2920, 1757, 1610, 1591, 1522, 1502, 1348; Mass spectrum (EI) m/z 327 (M^+). Exact mass calcd for $C_{16}H_{13}N_3O_3S$: m/z 327.0678. Found 327.0666.

3-Butyl-5-isobutyl-2-thioxo-imidazolidin-4-one (**4j**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.11 ~ 4.07 (m, 1H), 3.79 (t, J = 7.8 Hz, 2H), 1.82 ~ 1.75 (m, 2H), 1.71 ~ 1.53 (m, 3H), 1.41 ~ 1.31 (m, 2H), 0.99 (d, J = 6.0, 3.3 Hz, 6H), 0.95 ~ 0.92 (m, 3H), –NH proton signal was not observed.; ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.1, 174.6, 57.8, 41.2, 40.3, 29.7, 25.3, 23.0, 21.6, 20.0, 13.7; IR (cm^{-1}, neat): 3190, 2956, 2911, 1749, 1524, 1438; Mass spectrum (EI) m/z 228 (M^+). Exact mass calcd for $C_{11}H_{20}N_2OS$: m/z 228.3554. Found 228.1291.

5-Isobutyl-2-thioxo-3-m-tolyl-imidazolidin-4-one (**4k**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (bs, –NH), 7.43 ~ 7.38 (m, 1H), 7.27 (d, J = 7.8 Hz, 1H), 7.12 ~ 7.10 (m, 2H), 4.32 ~ 4.29 (m, 1H), 2.42 (s, 3H), 1.88 ~ 1.84 (m, 2H), 1.76 ~ 1.74 (m, 1H), 0.99 (t, J = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.8, 174.2, 139.4, 132.5, 130.3, 129.1, 128.8, 125.4, 58.6, 40.5, 25.2, 23.2, 21.7, 21.5; IR (cm^{-1}, neat): 2958, 2920, 1758, 1610, 1587, 1517, 1470; Mass spectrum (EI) m/z 262 (M^+). Exact mass calcd for $C_{14}H_{18}N_2OS$: m/z 262.1140. Found 262.1130.

3-(4-Fluoro-phenyl)-5-isobutyl-2-thioxo-imidazolidin-4-one (**4l**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.45 (bs, –NH), 7.33 ~ 7.28 (m, 2H), 7.23 ~ 7.16 (m, 2H), 4.34 ~ 4.30 (m, 1H), 1.85 ~ 1.80 (m, 2H), 1.76 ~ 1.71 (m, 1H), 1.00 (t, J = 5.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.5, 173.9, 162.7 (d, $^1J_{CF}$ = 248 Hz), 130.2 (d, $^3J_{CF}$ = 9 Hz), 128.4 (d, $^4J_{CF}$ = 3 Hz), 116.3 (d, $^2J_{CF}$ = 23 Hz), 58.5, 40.5, 25.2, 23.1,

21.5; IR (cm^{-1}, neat): 3154, 2965, 2911, 1760, 1600, 1514, 1407; Mass spectrum (EI) m/z 266 (M^+). Exact mass calcd for $C_{13}H_{15}FN_2OS$: m/z 266.0889. Found 266.0898.

5-Isobutyl-3-phenyl-2-thioxo-imidazolidin-4-one (**4m**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.48 (bs, –NH), 7.56 ~ 7.42 (m, 3 H), 7.34 ~ 7.29 (m, 2 H), 4.33 ~ 4.29 (m, 1 H), 1.93 ~ 1.84 (m, 2 H), 1.78 ~ 1.68 (m, 1 H), 1.00 (t, J = 6.1 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.7, 174.1, 132.7, 129.3, 129.2, 128.3, 58.6, 40.5, 25.1, 23.1, 21.5; IR (cm^{-1}, neat): 3172, 2956, 2920, 1758, 1596, 1521, 1409; Mass spectrum (EI) m/z 248 (M^+) Exact mass calcd for $C_{13}H_{16}N_2OS$: m/z 248.0983. Found 248.1005.

5-Isobutyl-3-(4-nitro-phenyl)-2-thioxo-imidazolidin-4-one (**4n**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.36 (d, J = 8.8 Hz, 2 H), 8.18 (bs, –NH), 7.58 (d, J = 8.8 Hz, 2 H), 4.45 ~ 4.30 (m, 1 H), 1.95 ~ 1.85 (m, 2 H), 1.83 ~ 1.73 (m, 1 H), 1.02 (t, J = 5.1 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.3, 173.2, 147.6, 138.0 129.3, 124.4, 58.6, 40.5, 25.2, 23.1, 21.5; IR (cm^{-1}, neat): 3199, 2956, 2920, 1757, 1610, 1600, 1521, 1465, 1402; Mass spectrum (EI) m/z 293 (M^+) Exact mass calcd for $C_{13}H_{15}N_3O_3S$: m/z 293.0834. Found 293.0831.

General procedure for the synthesis of thioxotetrahydropyrimidinones **(8a–8n)**

The polymer support (1 g, 0.17 mmol) dissolved in 10 mL of dichloromethane was coupled with 3-chloropropionyl chloride (0.05 mL, 0.51 mmol) in microwave cavity for 4 min to obtained PEG support intermediate **5**. The reaction mixtures were then added ether to precipitate the PEG support intermediate , which was filtered and rinsed to remove un-reacted reagents and side products. Nucleophilic substitution of polymer immobilized chloropropionyl ester **5** with primary amines (0.51 mmol) to give compound **6** was carried out in 200 W in the microwave for 15 minutes. Following ether washing and drying, a mixture of polymer bound diamine **6** (600 mg) and phenyl isothiocyanate (2.2 equiv.) in 5 mL of dichloromethane was heated in the microwave cavity with an output of 90 W for 10 min. Upon completion of the reaction, ether (20 mL) was added to the reaction mixture to precipitate the PEG-bound thiourea compound **7**. The precipitate was then collected on a sintered glass funnel and thoroughly washed with diethyl ether (20 mL × 3) following filtration. Finally, the desired cyclized thioxotetrahydropyrimidinone **8** was released from the support by heating in the microwave cavity with an output at 150 W for 7 min with K_2CO_3 (3 equiv.) in dichloromethane. The combined filtrate was dried to offer the corresponding crude product.

1-Butyl-3-phenyl-2-thioxo-tetrahydro-pyrimidine-4-one (**8a**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.47 ~ 7.39 (m, 3H), 7.12 (d, J = 7.5 Hz, 2H), 4.01 (t, J = 7.6 Hz, 2H), 3.75 (t, J = 6.7 Hz, 2H), 2.92 (t, J = 6.8 Hz, 2H), 1.78 ~ 1.69 (m, 2H), 1.44 ~ 1.36 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.2, 166.4, 139.6, 129.4, 129.0, 128.4, 55.7, 45.0, 31.9, 28.7, 20.1, 13.9; IR (cm^{-1}, neat): 2911, 2866, 2362, 1713, 1506, 1367, 1286; Mass spectrum (EI) m/z 262 (M^+). Exact mass calcd for $C_{14}H_{18}N_2OS$: m/z 262.1140. Found 262.1130.

1-Butyl-3-(4-nitro-phenyl)-2-thioxo-tetrahydro-pyrimidine-4-one (**8b**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.31(d, J = 8.9 Hz, 2H), 7.31 (d, J = 8.9 Hz, 2H), 4.01 (t, J = 7.6 Hz, 2H), 3.80 (t, J = 6.6 Hz, 2H), 2.97 (t, J = 6.8 Hz, 2H), 1.83 ~ 1.70 (m, 2H), 1.46 ~ 1.36 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.9, 166.1, 130.8, 124.3, 115.4, 114.6, 55.5, 45.0, 32.0, 31.8, 20.1, 13.9; IR (cm^{-1}, neat): 2924, 2857, 1714, 1507, 1426, 1288, 1202, 1167; Mass spectrum (EI) m/z 307 (M^+). Exact mass calcd for $C_{14}H_{17}FN_2OS$: m/z 307.0991. Found 307.0997.

1-Butyl-3-naphthalen-1-yl-2-thioxo-tetrahydro-pyrimidine-4-one (**8c**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.91 (m, 2H), 7.57 ~ 7.46 (m, 4H), 7.32 (d, J = 7.3 Hz, 1H), 4.14 ~ 3.81 (m, 2H), 3.93 ~ 3.81 (m, 2H), 3.12 ~ 2.95 (m, 2H), 1.84 ~ 1.73 (m, 2H), 1.50 ~ 1.37 (m, 2H), 1.00 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.9, 166.4, 136.1, 134.3, 130.4, 129.0, 128.8, 127.5, 127.0, 126.1, 125.4, 122.0, 55.7, 45.1, 32.0, 28.7, 20.2, 13.9; IR (cm^{-1}, neat): 2917, 1714, 1600, 1426, 1202, 1167; Mass spectrum (EI) m/z 312 (M^+). Exact mass calcd for $C_{18}H_{20}N_2S$: m/z 312.1296. Found 312.1295.

3-Allyl-1-butyl-2-thioxo-tetrahydro-pyrimidine-4-one (**8d**). ^1H NMR (300 MHz, $CDCl_3$): δ 5.96 ~ 5.83 (m, 1H), 5.17 (dd, J = 13.3, 11.5 Hz, 2H), 4.94 (d, J = 5.3 Hz, 2H), 3.97 (t, J = 7.7 Hz, 2H), 3.58 (t, J = 6.8 Hz, 2H), 2.74 (t, J = 6.7 Hz, 2H), 1.73 ~ 1.63 (m, 2H), 1.43 ~ 1.33 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.4, 165.9,

133.0, 117.2, 55.9, 48.3, 44.6, 31.5, 28.6, 20.1, 13.9; IR (cm^{-1}, neat): 2925, 2857, 1705, 1507, 1313, 1192; Mass spectrum (EI) m/z 226 (M^+). Exact mass calcd for $C_{11}H_{18}N_2OS$: m/z 226.1140. Found 226.1140.

3-(4-Fluoro-phenyl)-1-isopropyl-2-thioxo-tetrahydro-pyrimidine-4-one (**8e**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.12 (d, J = 1.0 Hz, 2H), 7.10 (s, 2H), 5.79 ~ 5.63 (m, 1H), 3.62 (t, J = 6.5 Hz, 2H), 2.86 (t, J = 6.9 Hz, 2H), 1.29 (d, J = 6.8 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.9, 166.6, 163.7(d, $^1J_{CF}$ = 246 Hz), 135.4 (d, $^4J_{CF}$ = 3 Hz), 131.1 (d, $^3J_{CF}$ = 9 Hz), 116.0 (d, $^2J_{CF}$ = 23 Hz), 53.4, 37.9, 32.1, 19.1; IR (cm^{-1}, neat): 2353, 2335, 1713; Mass spectrum (EI) m/z 266 (M^+). Exact mass calcd for $C_{13}H_{15}FN_2OS$: m/z 266.0889. Found 266.0872.

1-Isopropyl-3-(4-nitro-phenyl)-2-thioxo-piperidin-4-one (**8f**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 8.5 Hz, 2H), 7.29 (d, J = 9.0 Hz, 2H), 5.70 ~ 5.61 (m, 1H), 3.66 (t, J = 6.9 Hz, 2H), 2.89 (t, J = 6.9 Hz, 2H), 1.30 (d, J = 15.8 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.9, 166.6, 160.5, 135.4, 131.1, 131.0, 116.1, 115.8, 53.4, 37.9, 32.1, 19.1; IR (cm^{-1}, neat): 2920, 2848, 1722, 1524, 1492, 1345, 1286, 1200, 1164; Mass spectrum (EI) m/z 293 (M^+). Exact mass calcd for $C_{13}H_{15}N_3O_3S$: m/z 293.0834. Found 293.0847.

1-Furan-2-ylmethyl-2-thioxo-3-m-tolyl-tetrahydro-pyrimidin-4-one (**8g**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.42 (s, 1H), 7.34 ~ 7.28 (m, 1H), 7.25 ~ 7.20 (m, 1H), 6.95 (m, 2H), 6.45 (m, 1H), 6.39 ~ 6.38 (m, 1H), 5.27 (s, 2H), 3.80 (t, J = 6.9 Hz, 2H), 2.89 (t, J = 6.9 Hz, 2H), 2.38(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.8, 166.4, 148.8, 142.9, 139.5, 138.9, 129.8, 129.3, 128.8, 126.3, 110.7, 110.1, 51.0, 44.2, 31.8, 21.4; IR (cm^{-1}, neat): 2360, 1716, 1504, 1448, 1366, 1288, 1190; Mass spectrum (EI) m/z 300 (M^+). Exact mass calcd for $C_{16}H_{16}N_2O_2S$: m/z 300.0932. Found 300.0934.

3-Benzyl-1-furan-2-ylmethyl-2-thioxo-tetrahydro-pyrimidin-4-one (**8h**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.40 ~ 7.26 (m, 6H), 6.42 (d, J = 3.2 Hz, 1H), 6.37 ~ 6.36 (m, 1H), 5.60 (s, 2H), 5.24 (s, 2H), 3.62 (t, J = 2.6 Hz, 2H), 2.75 (t, J = 3.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.5, 166.2, 148.9, 142.8, 137.6, 128.3, 128.0, 127.2, 110.7, 109.9, 55.5, 49.2, 43.8, 31.5; IR (cm^{-1}, neat): 2924, 2345, 1707, 1451, 1371, 1183, 1150, 1074, 1012; Mass spectrum (EI) m/z 300 (M^+). Exact mass calcd for $C_{16}H_{16}N_2O_2S$: m/z 300.0932. Found 300.0921.

1-Thiophen-2-ylmethyl-2-thioxo-3-m-tolyl-tetrahydro-pyrimidin-4-one (**8i**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.37 ~ 7.20 (m, 3H), 7.14 (d, J = 3.3 Hz, 1H), 7.01 ~ 6.94 (m, 3H), 5.44 (s, 2H), 6.74 (t, J = 6.8 Hz, 2H), 2.87 (t, J = 6.8 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.8, 166.2, 139.5, 138.9, 137.3, 129.8, 129.4, 128.7, 127.8, 126.8, 126.4, 126.3, 53.2, 43.7, 31.9, 21.4; IR (cm^{-1}, neat): 2916, 2366, 1715, 1499, 1366, 1207; Mass spectrum (EI) m/z 316 (M^+). Exact mass calcd for $C_{16}H_{16}N_2O_2$: m/z 316.0704. Found 316.0704.

3-Benzyl-1-thiophen-2-ylmethyl-2-thioxo-tetrahydro-pyrimidin-4-one (**8j**). ^1H NMR (300 MHz, $CDCl_3$): δ 7.37 ~ 7.35 (m, 1H), 7.31 ~ 7.28 (m, 2H), 7.26 ~ 7.20 (m, 3H), 7.08 (d, J = 3.4 Hz, 1H), 6.99 ~ 6.96 (m, 1H), 5.62 (s, 2H), 5.41 (s, 2H), 3.59 (t, J = 6.9 Hz, 2H), 2.73 (t, J = 7.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.4, 166.0, 137.6, 137.4, 128.3, 128.0, 127.6, 126.7, 126.3, 114.6, 53.7, 49.2, 43.3, 33.6; IR (cm^{-1}, neat): 2360, 1706, 1496, 1450, 1372, 1186; Mass spectrum (EI) m/z 316 (M^+). Exact mass calcd for $C_{16}H_{16}N_2OS_2$: m/z 316.0704. Found 316.0699.

3-Allyl-1-(3-morpholin-4-yl-propyl)-2-thioxo-tetrahydro-pyrimidin-4-one (**8k**). ^1H NMR (300 MHz, $CDCl_3$): δ 5.94 ~ 5.81 (m, 1H), 5.25 ~ 5.12 (m, 2H), 4.93 ~ 4.91 (m, 2H), 4.00 (t, J = 7.3 Hz, 2H), 3.69 (t, J = 4.6 Hz, 4H), 3.61 (t, J = 6.7 Hz, 2H), 2.74 (t, J = 7.0 Hz, 2H), 2.45 ~ 2.37 (m, 6H), 1.99 ~ 1.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.5, 165.8, 132.9, 117.2, 66.9, 55.7, 54.3, 53.6, 48.2, 45.0, 31.5, 23.4; IR (cm^{-1}, neat): 2951, 2854, 2811, 2359, 1705, 1508, 1426, 1375, 1320, 1275, 1194, 1116, 1068, 917; Mass spectrum (EI) m/z 297 (M^+). Exact mass calcd for $C_{14}H_{23}N_3O_2S$: m/z 297.1511. Found 297.1507.

3-Allyl-1-pyridin-3-ylmethyl-2-thioxo-tetrahydro-pyrimidin-4-one (**8l**). ^1H NMR (300 MHz, $CDCl_3$): δ 8.60 (bs, 2H), 7.79 (d, J = 7.7 Hz, 1H), 7.36 (dd, J = 7.8, 4.7 Hz, 1H), 6.00 ~ 5.87 (m, 1H), 5.32 (s, 2H), 5.26 ~ 5.09 (m, 2H), 4.99 (d, J = 5.6 Hz, 2H), 3.55 (t, J = 6.8 Hz, 2H), 2.73 (t, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.0, 165.4, 149.7, 149.1, 135.6, 132.6, 131.3, 123.9, 117.6, 56.0, 48.8, 43.8, 31.4; IR (cm^{-1}, neat): 2920, 1707, 1500, 1425, 1373, 1318, 1193, 1129; Mass spectrum (EI) m/z 261 (M^+). Exact mass calcd for $C_{13}H_{15}N_3OS$:

m/z 261.0936. Found 261.0932.

3-Phenyl-1-pyridin-3-ylmethyl-2-thioxo-tetrahydro-pyrimidin-4-one (**8m**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (bd, 2H), 7.91 (d, J= 7.8 Hz, 1H), 7.51 ~ 7.36 (m, 4H), 7.18 ~ 7.15 (m, 2H), 5.36 (s, 2H), 3.75 (t, J = 6.8 Hz, 2H), 2.91 (t, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.7, 165.9, 149.8, 149.2, 139.6, 135.8, 131.3, 129.2, 129.1, 128.5, 124.0, 55.8, 44.1, 31.8; IR (cm^{-1}, neat): 2920, 2353, 2291, 1722, 1502, 1367, 1205, 1092; Mass spectrum (EI) m/z 297 (M^+). Exact mass calcd for $C_{16}H_{15}N_3OS$: m/z 297.0936. Found 297.0929.

1-Pyridin-3-ylmethyl-2-thioxo-3-m-tolyl-tetrahydro-pyrimidin-4-one (**8n**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (bd, 2H), 7.89 (d, J = 7.56 Hz, 1H), 7.39 ~ 7.34 (m, 2H), 7.23 (d, J = 7.56 Hz, 1H), 6.82 (m, 2H), 5.36 (s, J = 6.7 Hz, 2H), 3.73 (t, J = 6.7 Hz, 2H), 2.90 (t, J = 6.8 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.7, 165.9, 149.8, 149.2, 139.4, 139.0, 135.8, 131.3, 129.7, 129.4, 128.8, 126.2, 123.9, 55.8, 44.1, 31.8, 21.4; IR (cm^{-1}, neat): 2911, 2848, 1722, 1497, 1456, 1367, 1088; Mass spectrum (EI) m/z 311 (M^+). Exact mass calcd for $C_{17}H_{17}N_3OS$: m/z 311.1092. Found 311.1093

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References

1. (a) Czarnik, A. W. and DeWitt, S. H., *A Practical Guide to Combinatorial Chemistry*, American Chemical Society, Washington, D.C. 1997.
   (b) Fenniri, H., *Combinatorial Chemistry*, Oxford University Press, Inc: New York. 2000.
   (c) Seneci, P., *Solid-Phase Synthesis and Combinatorial Technologies*, John Wileys & Sons, Inc: New York. 2000.
2. Franzén, R., J. Comb. Chem., 2 (2000) 195–214.
3. (a) Sun, C. M., Comb. Chem. High Throughput Screening, 2 (1999) 299–309.
   (b) Toy, P. H. and Janda, K. D., Acc. Chem. Res., 33 (2000) 546–554.
   (c) Sun, C. M., 'Soluble Polymer-Supported Synthesis of Heterocyclic Libraries', in L. Bellavance (ed)., *'Combinatorial Chemistry Methods and Protocols', Methods in Molecular Biology Series*, The Humana Press Inc., New Jersey, (2002) Chapter 10, 345–371.
4. Shey, J. Y. and Sun, C. M., Tetrahedron Lett., 43 (2002) 1725–1729.
5. Brouillette, W. J., Jesitkov, V. P., Brown, M. L., Akhtar, M. S., DeLorey, T. M. and Brown, G. B. J., Med. Chem., 37 (1994) 3289–3293.
6. Scicinski, J. J., Barker, M. D., Murray, P. J. and Jarvie, E. M., Bioorg. Med. Chem. Lett., 8 (1998) 3609–3614.
7. Ooz, E., Somsak, L., Szilagyi, L., Kovacs, L., Docsa, T., Toth, B. and Gergely, P., Bioorg. Med. Chem. Lett., 9 (1999) 1385–1390.
8. Kuang, R., Epp, J. B., Ruan, S., Yu, H., Huang, P., He, S., Tu, J., Schechter, N. M., Turboy, J., Froelich, C. J. and Groutas, W. C. J., Am. Chem. Soc., 121 (1999) 8128–8129.
9. Kuang, R., Epp, J. B., Ruan, S., Chong, L. S., Venkataraman, R., Tu, J., He, S., Truong, T. M. and Groutas, W. C., Bioorg. Med. Chem., 8 (2000) 1005–1016.
10. He, S., Kuang, R., Venkataraman, R., Tu, J., Truong, T. M., Chan, H. T. and Groutas, W. C., Bioorg. Med. Chem., 8 (2000) 1713–1717.
11. Soliman, R. J., Med. Chem., 22 (1979) 321–325.
12. For reviews of microwave-assisted reactions in solution phase and solvent free condition: (a) Caddick, S., Tetrahedron, 51 (1995) 10403–10432.
    (b) Galema, S. A., Chem. Soc. Rev., 26 (1997) 233–238.
    (c) Varma, R. S., Green Chem., 1 (1999) 43–55.
    (c) Loupy, A. and Perreux, L., Tetrahedron, 57 (2001) 9199–9223.
    (d) Lidström, P., Tierney, J., Wathey, B. and Westman, J., Tetrahedron, 57 (2001) 9225–9283.
13. (a) Larhed, M. and Hallberg, A., Drug Discovery Today, 6 (2001) 406–416.
    (b) Wathey, B., Tierney, J., Lidström, P. and Westman, J., Drug Discovery Today, 7 (2002) 373–380.
14. Microwave-assisted solid-phase combinatorial synthesis: (a) Stadler, A. and Kappe, C. O. J., Comb. Chem., 3 (2001) 624–630.
    (b) Lew, A., Krutzik, P. O., Hart, M. E. and Chamberlin, R. A. J., Comb. Chem., 4 (2002) 95–105.
    (c) Swamy, K. M. K., Yeh, W. B., Lin, M. J. and Sun, C. M., Curr. Med. Chem., 10 (2003) 2403–2424.
15. DeWit, S. W., Kiely, J. S., StanKovic, C. J., Schroeder, M. C., Reynolds Cody, D. M. and Pavia, M. R., Proc. Natl. Acad. Sci., 90 (1993) 6909–6913.
16. Dressman, B. A., Spangle, L. A. and Kaldor, S. W., Tetrahedron Lett., 37 (1996) 937–940.
17. Sim, M. M., Lee, C. L. and Ganesan, A. J., Org. Chem., 62 (1997) 9358–9360.
18. Kim, S. W., Ahn, S. Y., Koh, J. S., Lee, J. H., Ro, S. and Cho, H. Y., Tetrahedron Lett., 38 (1997) 4603–4606.
19. Wu, S. and Janusz, J. M., Tetrahedron Lett., 41 (2000) 1165–1169.
20. Albericio, F., Garcia, J., Michelotti, E. L., Nicolas, E. and Tice, M., Tetrahedron Lett., 41 (2000) 3161–3163.
21. Albericio, F., Bryman, L. M., Garcia, J., Michelotti, E. L., Nicolas, E. and Tice, C. M. J., Comb. Chem., 3 (2001) 290–300.
22. Kita, R., Svec, F. and Fréchet, J. M. J., J. Comb. Chem., 3 (2001) 564–571.
23. Tremblay, M., Voyer, N., Boujabi, S. and Dewynter, G. F., J. Comb. Chem., 4 (2002) 429–435.
24. Lamothe, M., Lamuzzel, M. and Perez, M. J., Comb. Chem., 4 (2002) 73–78.
25. Nefzi, A., Giulianotti, M., Truong, L., Rattan, S., Ostresh, J. M. and Houghten, R. A. J., Comb. Chem., 4 (2002) 175–178.
26. Hanessian, S. and Yang, R. Y., Tetrahedron Lett., 37 (1996) 5835–5838.

27. Boeijen, A., Kruijtzer, J. A. W. and Liskamp, R. M. J., Bioorg. Med. Chem. Lett., 8 (1998) 2375–2380.
28. Park, K. H. and Kurth, M. J., Tetrahedron Lett., 41 (2000) 7409–7413.
29. Larnothe, M., Lannuzel, M. and Perez, M. J., Comb. Chem., 4 (2002) 73–78.
30. Gon, Y. D., Sohn, H. Y. and Kurth, M. J., J. Org. Chem., 63 (1998) 4854–4856.
31. Muccioli, G. G., Poupaert, J. H., Wouters, J., Norberg, B., Poppitz, W., Scriba, G. K. E. and Lambert, D. M., Tetrahedron, 59 (2003) 1301–1307.
32. Traceless solid-phase synthesis: Blaney, P., Grigg, R. and Sridharan, V. Chem. Rev., 102 (2002) 2607–2624